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TITLE: LIMITED TOXICITY AND MUTAGENICITY TESTING OF FIVE UNICHARGE PROPELLANT COMPOUNDS

SUBTITLE: Evaluation of Two Unicharge Propellants in the Primary Dermal Irritation Study

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13. ABSTRACT *(Maximum 200 words)*

Bis (2,2-dinitropropyl) acetal/formal (~50/50 mixture) ± diphenyl amine stabilizer (BDNPA/F±DPA) were tested for dermal irritation. One group of six rabbits per study were dermally exposed to the test article for four hours. Animals were observed for erythema and edema at 30-60 minutes, 24, 48 and 72 hours after dosing. Based upon the results of these assays, BDNPA/F+DPA and BDNPA/F-DPA were determined to be non-irritants. (Primary Dermal Irritation Index for both test articles=0.0) The Dermal Irritation Toxicity Category for both test articles is Class IV.

14. SUBJECT TERMS

Dermal irritation, unicharge, propellants, bis-(2,2-dinitropropyl)acetal, bis-(2,2-dinitropropyl) formal, lab animals, RAIII

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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__X__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

PI - Signature / Date 5/29/9?

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Evaluation of Two Unicharge Propellants in the Primary Dermal Irritation Study

EXECUTIVE SUMMARY

In order to assess the potential irritant and/or corrosive effects on the skin of rabbits, bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were applied to one intact skin site on each of six rabbits (3 males and 3 females) per study. No signs of erythema or edema were observed at any observation period in any animal receiving bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer or bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer. The studies were terminated following the 72 hour observation period.

Based upon the observations made in the Primary Dermal Irritation Study in rabbits, bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were determined to be non-irritants. (Primary Dermal Irritation Index for both test articles = 0.0). The Dermal Irritation Toxicity Category for both test articles is Class IV (mild or slight irritation at 72 hours).

Evaluation of Two Unicharge Propellants
in the Primary Dermal Irritation Study

PH 420-US-001, 002-91

STUDY DESCRIPTION

Sponsor:	U.S. Army Medical Research and Development Laboratory Fort Detrick Frederick, MD 21702-5010
Testing Facility:	Pharmakon Research International, Inc. P.O. Box 609 Waverly, PA 18471
Test Facility Study Conduct S.O.P. No.:	PH-420
Study Numbers:	PH 420-US-001-91 PH 420-US-002-91
Purpose of the Study:	To determine the potential irritant and/or corrosive effects on skin of rabbits.
Ownership of the Study:	The sponsor owns the study. All raw data, analyses and reports are the property of the sponsor.
Study Monitor:	Major Nathaniel Powell, U.S. Army Medical Research and Development Laboratory
Study Director:	Victor T. Mallory, B.S., RLAT, Pharmakon Research International, Inc.
Technical Performance:	Thomas O'Neill, B.S., LAT and Kim DiLeo, B.S., LAT
Q.A.U. Responsible Personnel:	Leslie J. Pinnell, M.S.
Date Study Director Signed Protocols:	September 23, 1991

Evaluation of Two Unicharge Propellants
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Dates of Technical Performance:
PH 420-US-001-91 - December 4, 1991 through December 7, 1991
PH 420-US-002-91 - December 3, 1991 through December 6, 1991

Good Laboratory Practices Statement: These studies were conducted in compliance with the Good Laboratory Practice Regulations. There were no deviations from the GLP Regulations which affected the quality or integrity of the study. Q.A.U. findings from the inspections conducted of this study and from the audit of the final report are documented and have been provided to the study director and the test facility management.

Records Maintained: All raw data, final report documentation and protocol will be maintained in the archives of Pharmakon Research International, Inc.

Recordings: Standard Pharmakon Notebook

Notebook Reference: Notebook #1503, pages 197-198, 200-201

TEST ARTICLES

TEST ARTICLE	DESCRIPTION	LOT #	pH	CAS #	DATE SUBMITTED
bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer (BDNPA/F+DPA)	yellow liquid	Set #1	5	5108-69-0	9/19/91
bis-(2,2-dinitropropyl) formal without diphenyl amine stabilizer (BDNPA/F-DPA)	yellow liquid	Set #2	5	5917-61-3	9/19/91

Analysis of Purity: The purity, identity, strength and stability of the test articles were the responsibility of the sponsor.

Stability: There was no apparent change in the physical appearance of the test articles during administration.

TEST SYSTEM

Species:	Rabbit
Strain (Source):	CAMM Research Lab Animals, Wayne, NJ
Sex:	Male and female
Age at Initiation:	8-12 weeks
Weight Range:	1.751 - 2.795 kilograms
No. on Study:	Six (6) (three males and three females) per study.
Method and Justification for Randomization:	Selection of rabbits based upon body weight.
Acclimation Period:	Minimum of five (5) days
System of Identification:	Cage cards were marked with the study number, animal number, dose level and sex. Rabbits were ear tagged.

HUSBANDRY

Research Facility Registration:	U.S.D.A. Registration No. 23-R-107 under the Animal Welfare Act 74: SC 2131 *et seq*.
Animal Rooms:	Separate isolation by test system Light cycle - 12 hours light, 12 hours dark Temperature/Relative Humidity - Every attempt was made to maintain a temperature of 20°C ± 3°C (63-73°F) and a relative humidity of 30 to 70%. Any excursions outside the temperature or humidity ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.
Housing:	Rabbits were housed individually in cages, sized in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.
Sanitization:	Waste material was removed twice weekly. Cages and feeders were sanitized every two weeks.

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Food:	Purina Lab Rabbit Chow H.F.^{R}, ad libitum. Food was checked daily and added or replaced as needed. Feeders are designed to reduce soiling, bridging and scattering.
Food Analysis:	There were no contaminants that were reasonably expected to be present in the dietary material known to be capable of interfering with the purpose or conduct of the study.
Water:	Fresh tap water, ad libitum.
Water Analysis:	Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

METHODS

Rationale for Test System:	The albino rabbit is recommended as the preferred species.
Compound Preparation:	The test articles were dosed as received.
Dose Administration:	0.5 mL/site
Rationale for Dose Selection:	According to the EPA Federal Register, Vol. 50, No. 188, Friday, September 27, 1985
Route of Administration:	The test articles were applied directly on the intact skin site.
Rationale for Route of Administration:	According to the EPA Federal Register, Vol. 50, No. 188, Friday, September 27, 1985
Frequency and Duration of Administration:	Administered once and remained in contact with the skin for four (4) hours.
No. of Animals Per Dose Group:	Six (6)
No. and Code of Dose Group:	Rabbit No. — Dose 5220-5225 — 0.5 mL/site [bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer] 5201-5206 — 0.5 mL/site [bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer]
Length of Studies:	Seventy-two (72) hours

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Method of Study Performance:

Approximately 24 hours before the test, fur was removed from the test area by clipping from the dorsal area of the trunk of the animals. Care was taken to avoid abrading the skin. The test substance was applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which was held in place with non-irritating tape. The patch was loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. The test substance was kept in contact with the skin site for four (4) hours. At the end of the four (4) hour exposure period, the wrappings were removed. Animals were observed for signs of erythema and edema and scored according to the Draize Scale at 30 to 60 minutes, 24, 48 and 72 hours following patch removal.

RESULTS

No signs of erythema or edema were observed at any observation period in any animal receiving bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer or bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer. The studies were terminated following the 72 hour observation period.

CONCLUSIONS

Based upon the observations made in the Primary Dermal Irritation Study studies in rabbits, bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were determined to be non-irritants. (Primary Dermal Irritation Index for both test articles = 0.0). The Dermal Irritation Toxicity Category for both test articles is Class IV (mild or slight irritation at 72 hours).

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Evaluation of Two Unicharge Propellants
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TABLE I

[1]Draize Evaluation of Dermal Irritation

I. Dermal Observations

Erythema and Eschar Formation (Most severely affected area graded):

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Edema Formation (Most severely affected area graded):

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

[1]Draize, J.H. 1959. The Appraisal of Chemicals in Foods, Drugs and Cosmetics, pp. 36-45. Association of Food and Drug Officials of the United States, Austin, Texas.

Federal Hazardous Substances Act Regulations. 16 CFR 1500.

Dermal Irritation
Toxicity Categories:

I	II	III	IV
Corrosive	Severe Irritation at 72 hours	Moderate Irritation at 72 hours	Mild or Slight Irritation at 72 hours

Table II

Summary of Observations/Post-Treatment of Two Unicharge Propellants
in the Primary Dermal Irritation Study

PH 420-US-001, 002-91

Bis-(2,2-Dinitropropyl) Acetal with Diphenyl Amine Stabilizer

Rabbit No.	Sex	30-60 minutes		24 hours		48 hours		72 hours	
		Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
5220	M	0	0	0	0	0	0	0	0
5221	M	0	0	0	0	0	0	0	0
5222	M	0	0	0	0	0	0	0	0
5223	F	0	0	0	0	0	0	0	0
5224	F	0	0	0	0	0	0	0	0
5225	F	0	0	0	0	0	0	0	0

Table II (continued)

Summary of Observations/Post-Treatment of Two Unicharge Propellants in the Primary Dermal Irritation Study

PH 420-US-001, 002-91

Bis-(2,2-Dinitropropyl) Formal without Diphenyl Amine Stablizer

Rabbit No.	Sex	30-60 minutes		24 hours		48 hours		72 hours	
		Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
5201	M	0	0	0	0	0	0	0	0
5202	M	0	0	0	0	0	0	0	0
5203	M	0	0	0	0	0	0	0	0
5204	F	0	0	0	0	0	0	0	0
5205	F	0	0	0	0	0	0	0	0
5206	F	0	0	0	0	0	0	0	0

Table III. Summary of Body Weights (g) of Two Unicharge Propellants in the Primary Dermal Irritation Study

PH 420-US-001, 002-91

Bis-(2,2-Dinitropropyl) Acetal with Diphenyl Amine Stabilizer

Animal Number	Sex	Initial	Final
5220	M	2795	2863
5221	M	1782	1904
5222	M	2337	2415
5223	F	1751	1863
5224	F	2124	2211
5225	F	2169	2274

Bis-(2,2-Dinitropropyl) Formal without Diphenyl Amine Stablizer

Animal Number	Sex	Initial	Final
5201	M	2170	2225
5202	M	2018	2164
5203	M	1854	2009
5204	F	2143	2258
5205	F	2072	2166
5206	F	1981	2085

QUALITY ASSURANCE UNIT STATEMENT

Study Nos.: PH 420-US-001-91
PH 420-US-002-91

Study Director: Victor T. Mallory

The Quality Assurance Unit conducted the inspections listed below and reported the results to the study director and to management on the dates indicated.

The following inspections were performed:

Interval	Date
In Life Phase	December 3, 1991 December 4, 1991
Reporting Phase	January 29, 1992

Date QAU Report Issued

To Study Director	To Management
January 29, 1992	January 29, 1992

Quality Assurance

Date Jan 29, 1992

COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practices (GLP) as promulgated by the following regulatory agencies.

EPA as stated in the Federal Register, 40 CFR Parts 160 and 792.

Organization for Economic Co-operation and Development Guidelines for Testing Chemicals (OECD), ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12, 1981.

U.S. Food and Drug Administration as stated in 58 CFR Part 21.

Study Nos.: PH 420-US-001-91
PH 420-US-002-91

To the best of my knowledge, this study was conducted in accordance with applicable Good Laboratory Practice regulations; there were no deviations from these regulations that impacted on study conclusions.

Study Director

Date May 29, 1995